UK NEQAS
Leucocyte Immunophenotyping

Sheffield Teaching Hospitals NHS
NHS Foundation Trust

Paroxysmal Nocturnal Haemoglobinuria

Participant: 42981 **Trial No: 151602**

Date Issued: 07-September-2015 **Closing Date: 25-September-2015**

Your Results

Sample: 079

Trial Comments

This trial was issued to 153 participants

Sample Comment
This sample was manufactured from stabilised whole blood spiked with stabilised PNH material from a consented patient.

Target Cells	Your Result	Median Result	Lower Quartile	Upper Quartile	Number of Returns
RBC PNH Clone - CD59		2.00	1.76	2.47	121
RBC Type II Cells - CD59		0.60	0.44	0.90	112
RBC Type III Cells - CD59		1.40	1.13	1.65	114
Granulocytes PNH Clone - CD24	2.50	3.70	3.36	3.97	97

Performance Monitoring

Sample 079

	Consensus	Your Result	Classification	Running Classification
RBC	Clone Present	Clone Absent	Outwith Consensus	Red 3
WBC	Clone Present	Clone Present	Within Consensus	Red 3

1 = All Samples Within Consensus across last 3 samples
2 = 1 Sample Outwith Consensus across last 3 samples
3 = 2 or more Samples Outwith Consensus across last 3 samples
= Not enough samples to determine Running Consensus

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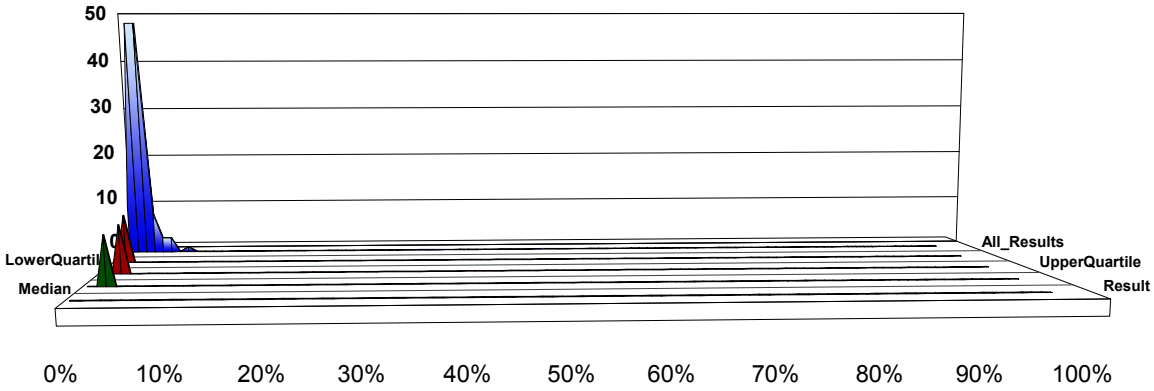
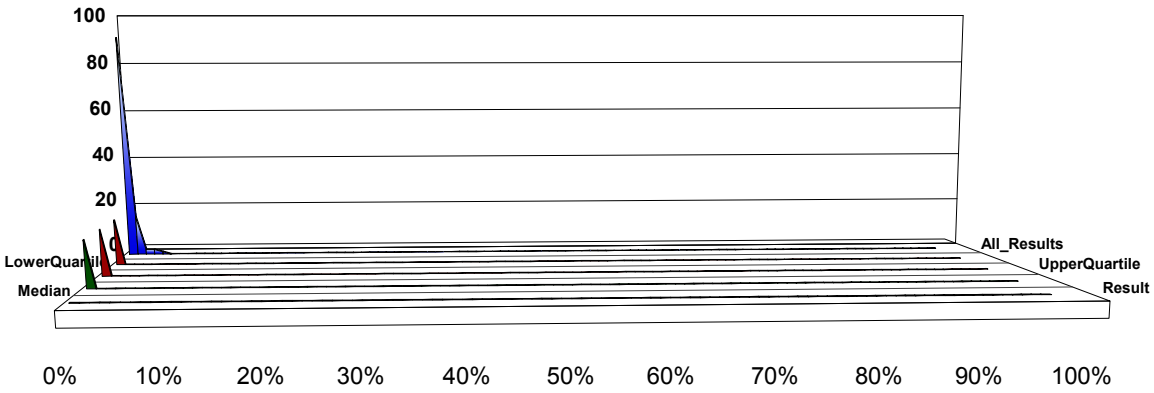
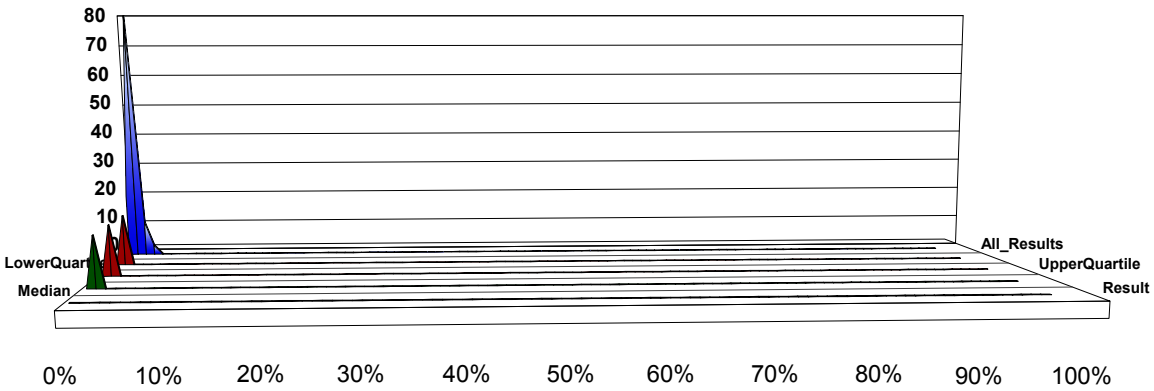
RBC PNH Clone - CD59



RBC Type II Cells - CD59



RBC Type III Cells - CD59



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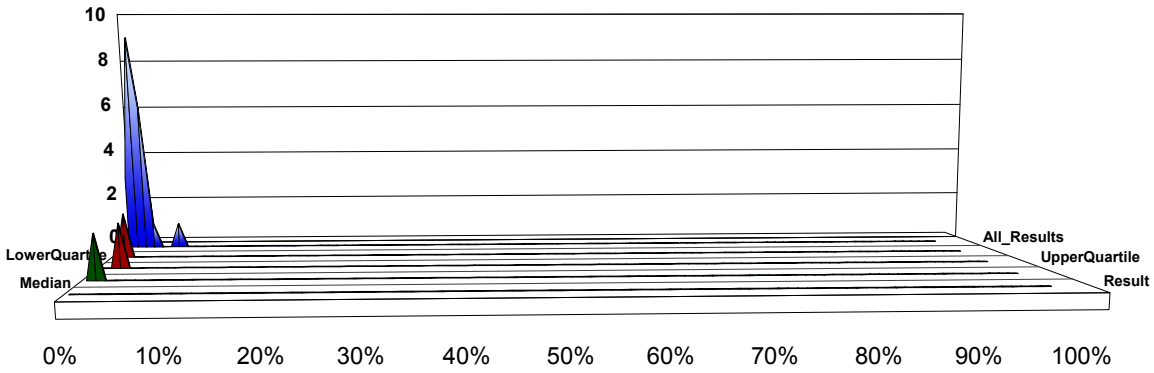
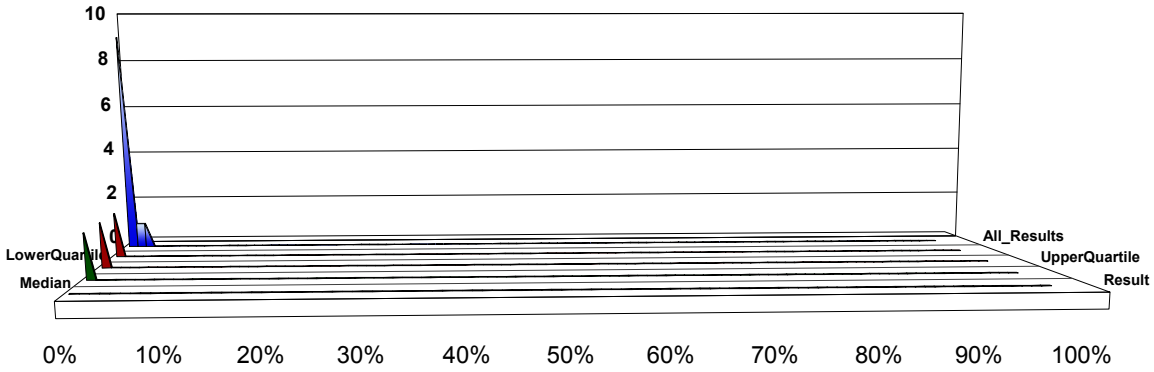
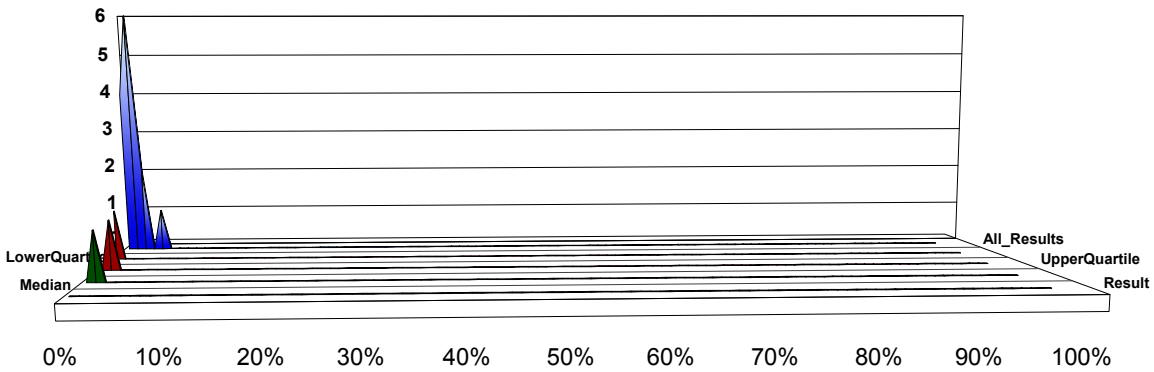
RBC PNH Clone - CD55



RBC Type II cells - CD55



RBC Type III cells - CD55



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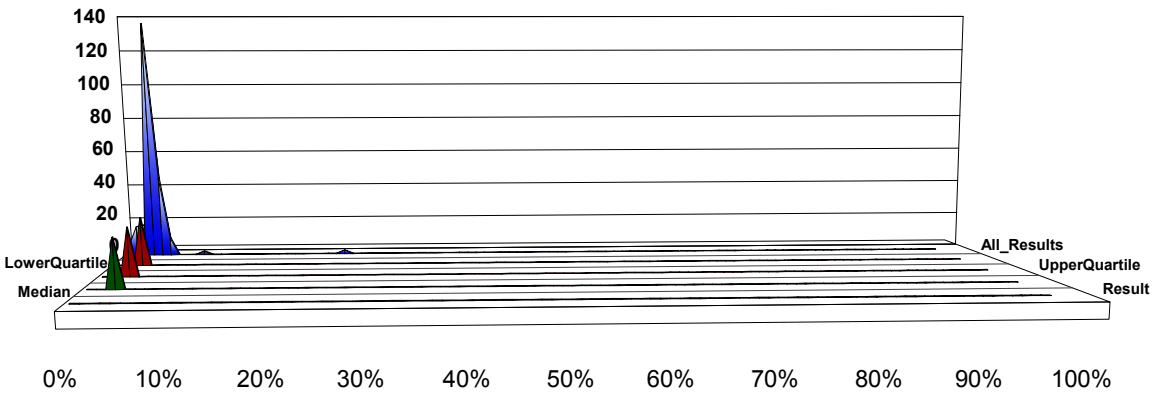
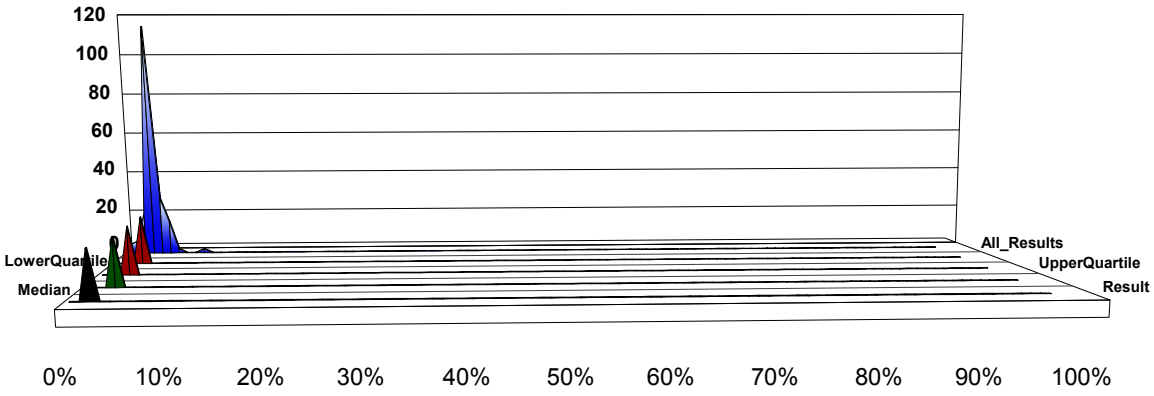
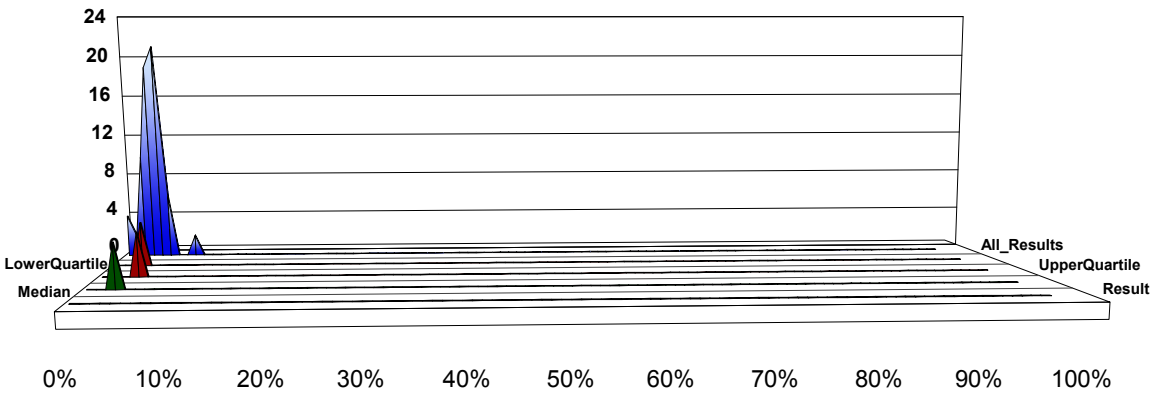
Granulocytes PNH Clone - FLAER



Granulocytes PNH Clone - CD24



Granulocytes PNH Clone - CD16



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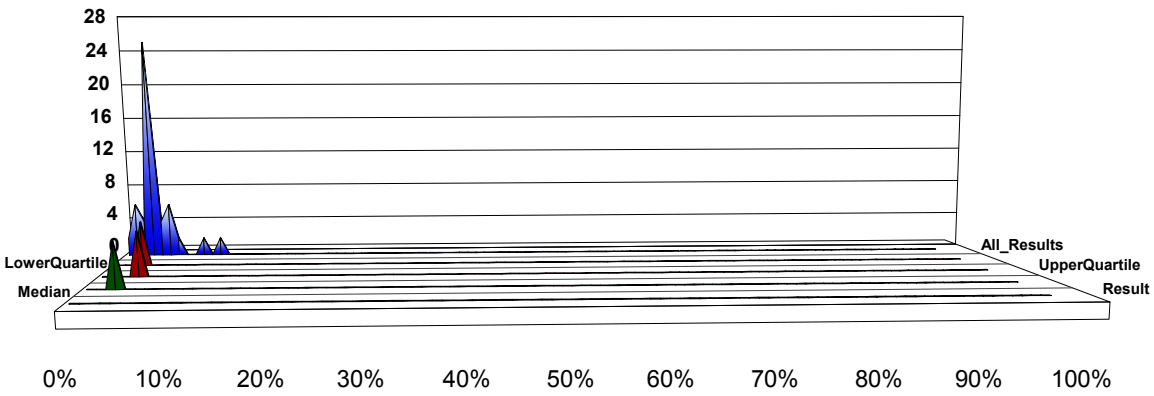
Granulocytes PNH Clone - CD66b



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Method Breakdown

RBC PNH Clone

Flow Cytometer	Median	Lower Quartile	Upper Quartile	Returns
CD55				
All	1.77	1.58	2.13	20
BD	1.73	1.44	2.23	16
CD59				
All	2.00	1.76	2.47	121
BD	2.00	1.70	2.40	85
Coulter	1.90	1.77	2.45	35

Antibody Manufacturer	Median	Lower Quartile	Upper Quartile	Returns
CD55				
All	1.77	1.58	2.13	20
BD Biosciences	1.94	1.35	2.28	10
CD59				
All	2.00	1.76	2.47	121
BD Biosciences	2.10	1.85	2.52	13
Beckman Coulter	1.72	1.37	1.79	10
eBioscience	2.22	1.96	2.54	12
invitrogen	2.09	1.80	2.52	61

Fluorochrome	Median	Lower Quartile	Upper Quartile	Returns
CD55				
All	1.77	1.58	2.13	20
PE	1.76	1.43	2.20	15
CD59				
All	2.00	1.76	2.47	121
PE	2.08	1.80	2.51	93
FITC	1.85	1.59	2.09	27

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Method Breakdown
Granulocytes PNH Clone

Flow Cytometer	Median	Lower Quartile	Upper Quartile	Returns
CD16				
All	3.90	3.26	4.35	33
BD	3.97	3.26	4.35	25
CD24				
All	3.70	3.36	3.97	97
BD	3.72	3.40	4.00	68
Coulter	3.52	3.34	3.81	28
CD55				
All	3.10	2.80	3.48	18
BD	3.10	2.70	3.65	11
CD59				
All	3.33	2.37	4.00	18
BD	3.40	2.30	4.00	11
CD66b				
All	3.70	3.30	4.20	27
BD	3.86	3.45	4.55	20
FLAER				
All	3.70	3.36	3.98	120
BD	3.70	3.40	4.00	81
Coulter	3.50	3.24	3.82	38

Antibody Manufacturer	Median	Lower Quartile	Upper Quartile	Returns
CD16				
All	3.90	3.26	4.35	33
BD Biosciences	4.00	3.51	4.53	16
Beckman Coulter	3.84	3.38	4.30	10
CD24				
All	3.70	3.36	3.97	97
BD Biosciences	3.71	3.45	3.89	27
Beckman Coulter	3.53	3.34	3.93	44
Pharmingen	3.75	3.35	4.15	20
CD55				
All	3.10	2.80	3.48	18
CD59				
All	3.33	2.37	4.00	18
CD66b				
All	3.70	3.30	4.20	27
FLAER				
All	3.70	3.36	3.98	120
Other	3.70	3.40	3.96	65
Protox	3.60	3.40	4.00	43

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Fluorochrome	Median	Lower Quartile	Upper Quartile	Returns
CD16				
All	3.90	3.26	4.35	33
CD24				
All	3.70	3.36	3.97	97
PE	3.70	3.35	3.90	73
CD55				
All	3.10	2.80	3.48	18
PE	3.23	2.88	3.43	12
CD59				
All	3.33	2.37	4.00	18
PE	4.00	3.42	4.00	10
CD66b				
All	3.70	3.30	4.20	27
FITC	3.70	3.30	3.90	19
FLAER				
All	3.70	3.36	3.98	120
FITC	3.70	3.50	3.98	31
AlexaFluor 488	3.70	3.35	3.97	89

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Paroxysmal Nocturnal Haemoglobinuria

Participant: 42981 **Trial No: 151602**

Date Issued: 07-September-2015 **Closing Date: 25-September-2015**

Your Results

Sample: 080

Trial Comments

This trial was issued to 153 participants

Sample Comment
This sample was manufactured from stabilised whole blood spiked with stabilised PNH material from a consented patient.

Target Cells	Your Result	Median Result	Lower Quartile	Upper Quartile	Number of Returns
RBC PNH Clone - CD59		0.80	0.67	1.00	120
RBC Type II Cells - CD59		0.27	0.17	0.40	112
RBC Type III Cells - CD59		0.50	0.40	0.60	114
Granulocytes PNH Clone - CD24	0.50	1.49	1.30	1.69	97

Performance Monitoring

Sample 080

	Consensus	Your Result	Classification	Running Classification
RBC	Clone Present	Clone Absent	Outwith Consensus	Red 3
WBC	Clone Present	Clone Present	Within Consensus	Amber 2

1 = All Samples Within Consensus across last 3 samples
2 = 1 Sample Outwith Consensus across last 3 samples
3 = 2 or more Samples Outwith Consensus across last 3 samples
= Not enough samples to determine Running Consensus

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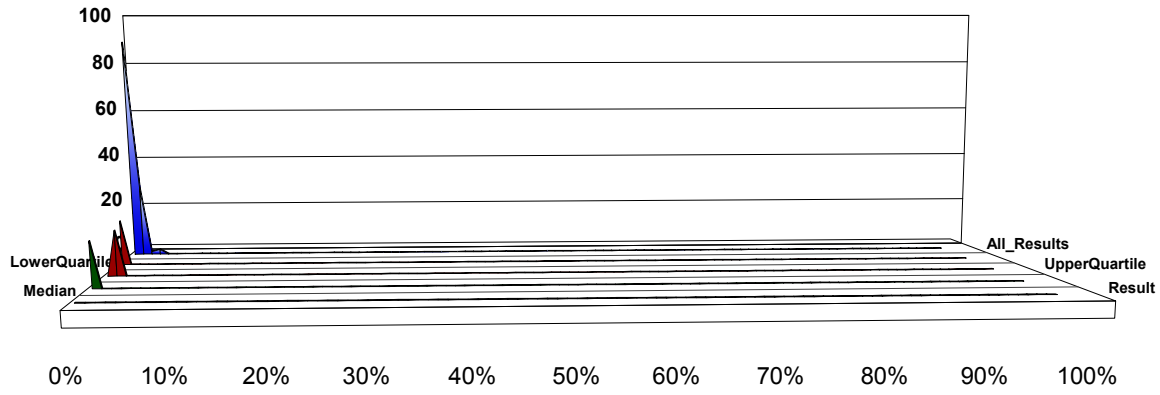
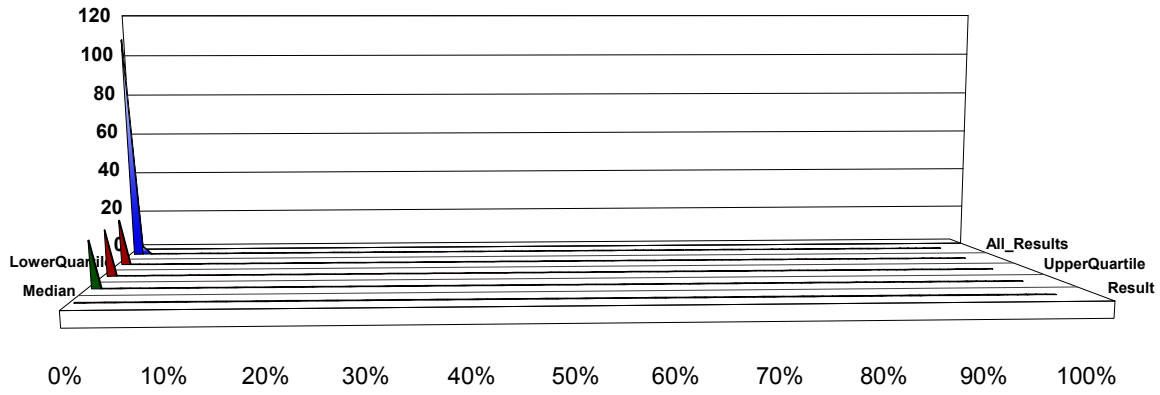
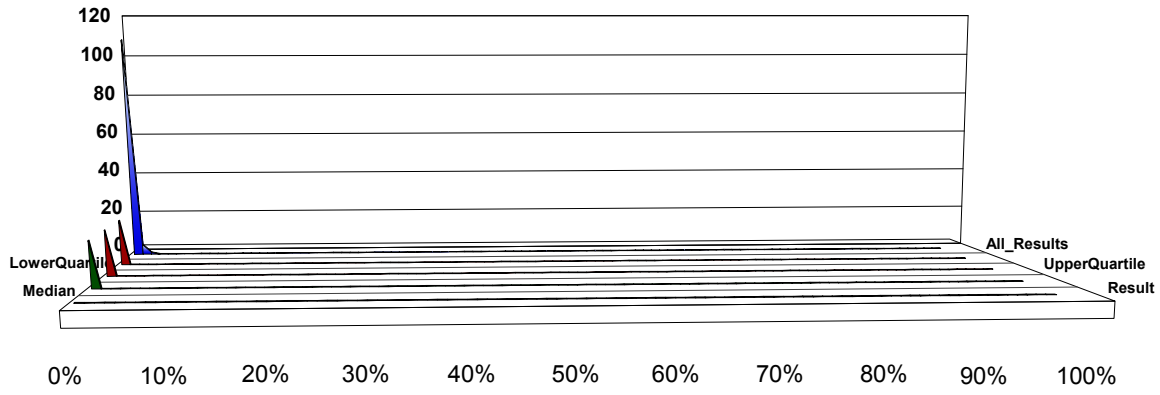
RBC PNH Clone - CD59



RBC Type II Cells - CD59



RBC Type III Cells - CD59



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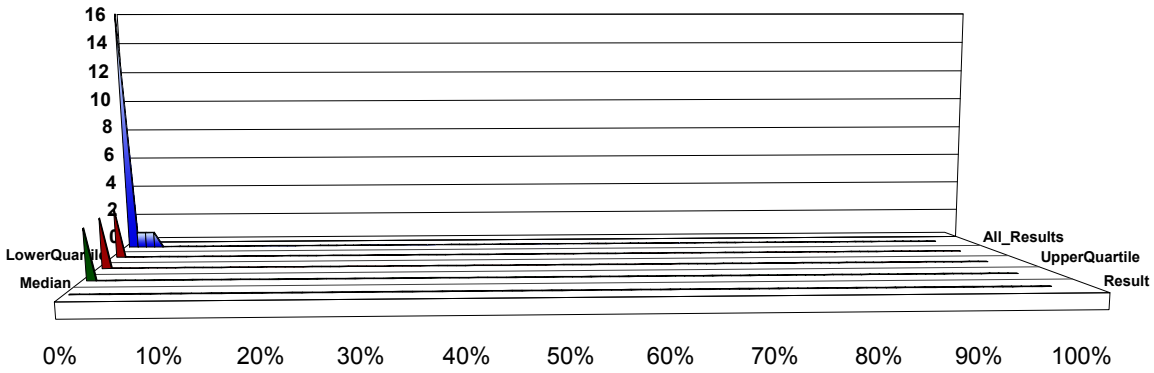
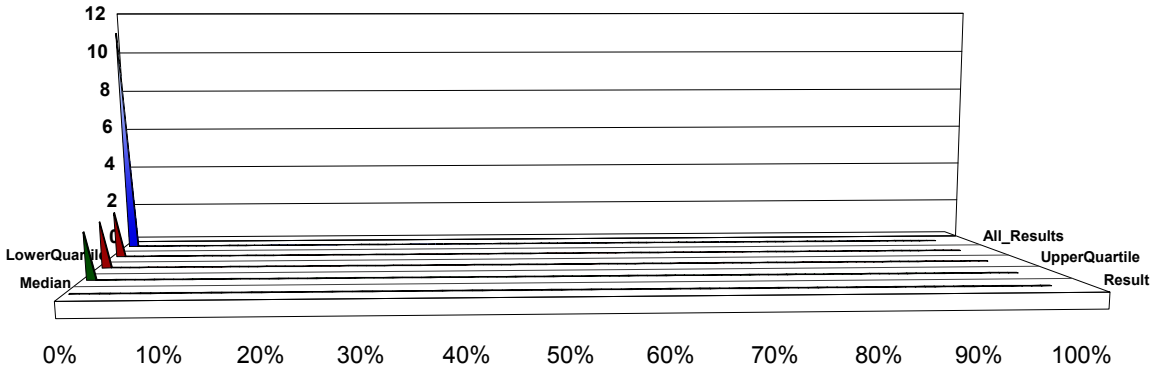
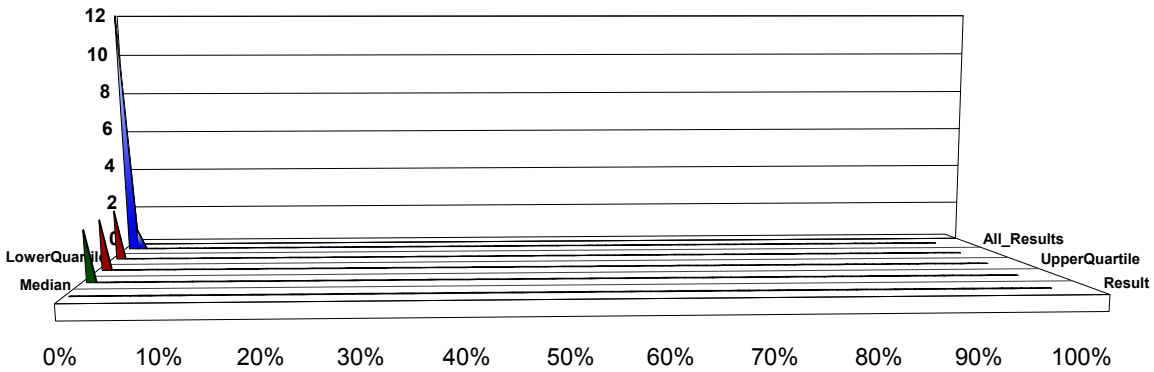
RBC PNH Clone - CD55



RBC Type II cells - CD55



RBC Type III cells - CD55



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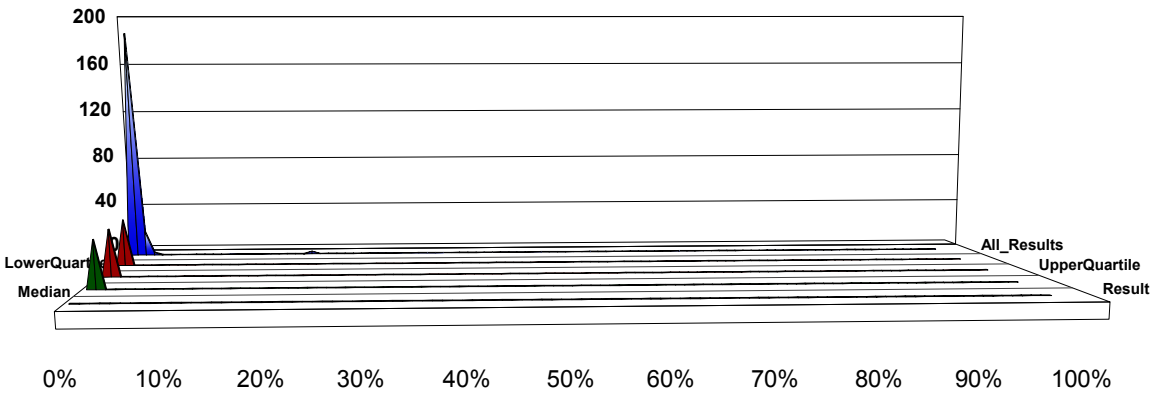
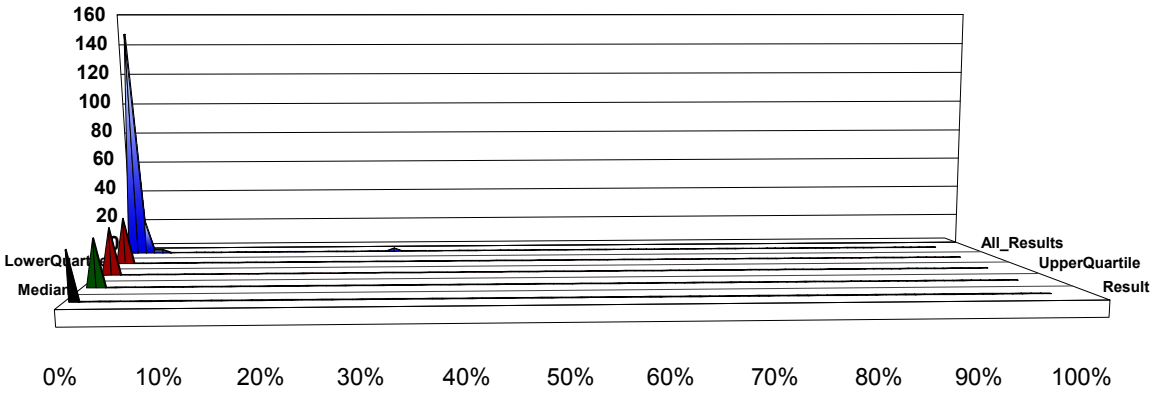
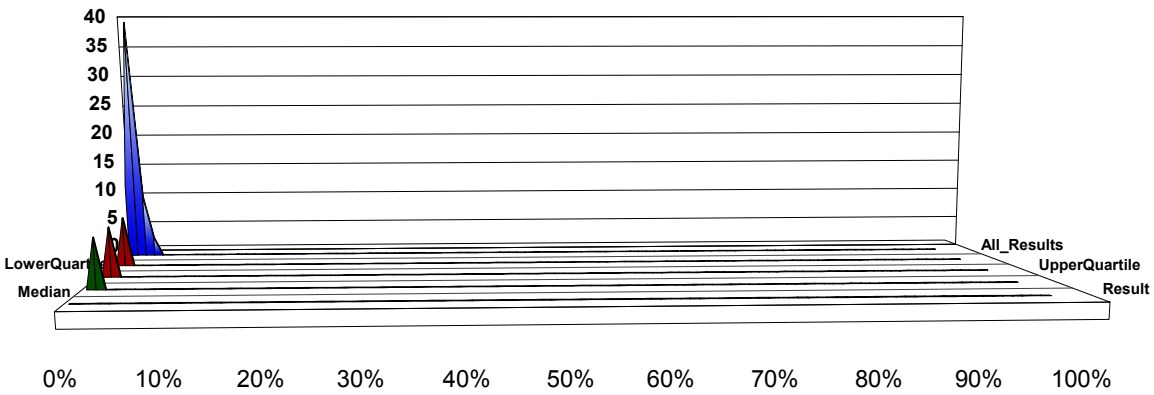
Granulocytes PNH Clone - FLAER



Granulocytes PNH Clone - CD24



Granulocytes PNH Clone - CD16



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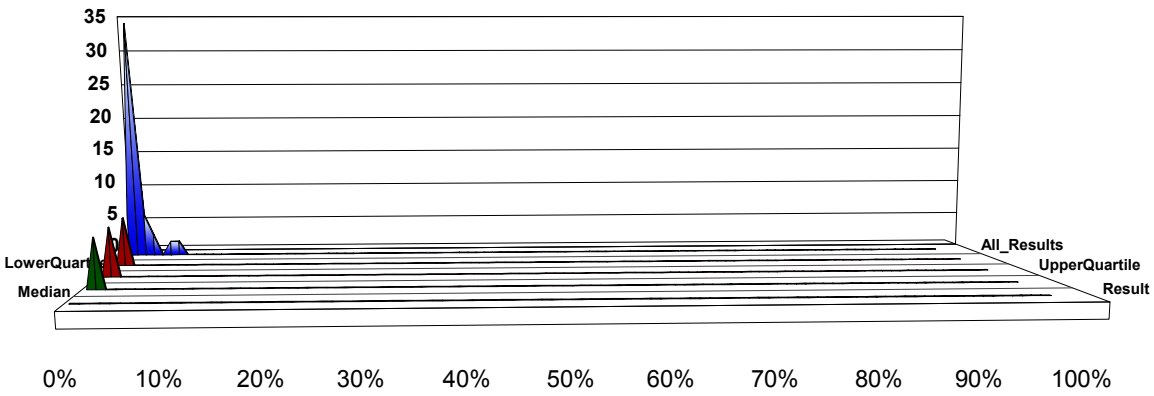
Granulocytes PNH Clone - CD66b



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Method Breakdown

RBC PNH Clone

Flow Cytometer	Median	Lower Quartile	Upper Quartile	Returns
CD55				
All	0.70	0.50	0.81	19
BD	0.69	0.46	0.86	15
CD59				
All	0.80	0.67	1.00	120
BD	0.80	0.70	1.00	84
Coulter	0.78	0.65	0.90	35

Antibody Manufacturer	Median	Lower Quartile	Upper Quartile	Returns
CD55				
All	0.70	0.50	0.81	19
CD59				
All	0.80	0.67	1.00	120
BD Biosciences	1.05	0.80	1.43	13
Beckman Coulter	0.64	0.56	0.78	10
eBioscience	0.85	0.73	1.07	12
invitrogen	0.80	0.69	0.92	61

Fluorochrome	Median	Lower Quartile	Upper Quartile	Returns
CD55				
All	0.70	0.50	0.81	19
PE	0.70	0.51	0.88	14
CD59				
All	0.80	0.67	1.00	120
PE	0.80	0.69	0.99	92
FITC	0.78	0.50	1.05	27

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Method Breakdown
Granulocytes PNH Clone

Flow Cytometer	Median	Lower Quartile	Upper Quartile	Returns
CD16				
All	1.50	1.20	1.70	33
BD	1.50	1.20	1.70	25
CD24				
All	1.49	1.30	1.69	97
BD	1.50	1.39	1.70	68
Coulter	1.33	1.18	1.54	28
CD55				
All	1.18	0.90	1.32	18
BD	1.20	0.80	1.40	11
CD59				
All	1.30	0.94	1.50	18
BD	1.30	0.53	1.55	11
CD66b				
All	1.50	1.15	1.90	27
BD	1.54	1.18	1.80	20
FLAER				
All	1.47	1.30	1.60	119
BD	1.50	1.37	1.69	80
Coulter	1.32	1.10	1.51	38

Antibody Manufacturer	Median	Lower Quartile	Upper Quartile	Returns
CD16				
All	1.50	1.20	1.70	33
BD Biosciences	1.49	1.20	2.03	16
Beckman Coulter	1.50	1.33	1.68	10
CD24				
All	1.49	1.30	1.69	97
BD Biosciences	1.49	1.40	1.63	27
Beckman Coulter	1.40	1.30	1.60	44
Pharmingen	1.55	1.19	1.70	20
CD55				
All	1.18	0.90	1.32	18
CD59				
All	1.30	0.94	1.50	18
CD66b				
All	1.50	1.15	1.90	27
FLAER				
All	1.47	1.30	1.60	119
Other	1.50	1.31	1.64	62
Protox	1.50	1.30	1.61	43

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Fluorochrome	Median	Lower Quartile	Upper Quartile	Returns
CD16				
All	1.50	1.20	1.70	33
CD24				
All	1.49	1.30	1.69	97
PE	1.50	1.30	1.66	73
CD55				
All	1.18	0.90	1.32	18
PE	1.18	0.98	1.31	12
CD59				
All	1.30	0.94	1.50	18
PE	1.50	1.35	1.64	10
CD66b				
All	1.50	1.15	1.90	27
FITC	1.50	1.15	1.60	19
FLAER				
All	1.47	1.30	1.60	119
FITC	1.50	1.38	1.63	31
AlexaFluor 488	1.46	1.30	1.60	87

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PNH 151602

Sample 079

This sample was PNH material spiked into a unit of normal whole blood; the median values of the results returned accurately reflected those obtained at pre-issue testing.

The consensus result for this sample was **Clone Present** for both Red Cell and Granulocyte clones. 4 centres did not identify a Red Cell PNH clone upon analysis all recording a 0% clone as clone absent, one other centre identified a 0.1% clone which was also reported clone absent. All of these were out of consensus.

Only 4 participants failed to identify the granulocyte clone on all of their markers and reported their results as being clone absent. One other centre identified a 1.7% clone and recorded this as being clone absent. Again, all of these were out of consensus.

7 centres did not return any results for this sample.

Sample 080

This sample was also PNH material spiked into a unit of normal whole blood; the median values of the results returned accurately reflected those obtained at pre-issue testing.

The consensus result for this sample was **Clone Present** for both Red Cell and Granulocyte clones. 6 centres did not identify a Red Cell PNH clone upon analysis all recording a 0% clone as clone absent, four of these centres also missed the red cell clone on sample 079. Additionally, two centres identified a clone (0.1% and 0.9% respectively) which were reported clone absent and out of consensus.

5 participants failed to identify the granulocyte clone on all of their markers and reported their results as being clone absent, four of these centres also missed the granulocyte clone on sample 079. One other centre identified a 0.8% clone and recorded this as being clone absent.

7 centres did not return results for this sample.

Please note that UK NEQAS for Leucocyte Immunophenotyping will be running Instrument specific PNH testing courses throughout the year, details of which are included at the end of this report.

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Information with respect to compliance with standards BS EN ISO/IEC 17043:2010

4.8.2 a) The proficiency testing provider for this programme is:
UK NEQAS for Leucocyte Immunophenotyping
Pegasus House, 4th Floor Suite
463A Glossop Road
Sheffield, S10 2QD
United Kingdom
Tel: +44 (0) 114 267 3600, Fax: +44 (0) 114 267 3601
e-mail: nicola.rose@ukneqasli.co.uk

4.8.2 b) The coordinators of UK NEQAS LI programmes are Prof David Barnett and Mr Liam Whitby.

4.8.2 c) Person(s) authorizing this report:
Prof David Barnett, Director or Mr Liam Whitby, Operations Manager of UK NEQAS LI

4.8.2 d) No activities in relation to this EQA exercise were subcontracted.

4.8.2 g) The UK NEQAS LI Confidentiality Policy can be found in the Quality Manual which is available by contacting the UK NEQAS LI office. Participant details, their results and their performance data remain confidential unless revealed to the relevant NQAAP when a UK participant is identified as having performance issues.

4.8.2 i) All EQA samples are prepared in accordance with strict Standard Operational Procedures by trained personnel proven to ensure homogeneity and stability. Where appropriate/possible EQA samples are tested prior to issue. Where the sample(s) issued is stabilised blood or platelets, pre and post stability testing will have proved sample suitability prior to issue.

4.8.2 l), n), o), r) & s) Please refer to the UK NEQAS LI website at www.ukneqasli.co.uk for detailed information on each programme including the scoring systems applied to assess performance. Where a scoring system refers to the 'consensus result' this means the result reported by the majority of participants for that trial issue. Advice on the interpretation of statistical analyses and the criteria on which performance is measured is also given. Please note that where different methods/procedures are used by different groups of participants these may be displayed within your report, but the same scoring system is applied to all participants irrespective of method/procedure used.

4.8.2 m) We do not assign values against reference materials or calibrants.

4.8.2 q) Details of the programme designs as authorized by The Steering Committee and Specialist Advisory Group can be found on our website at www.ukneqasli.co.uk. The proposed trial issue schedule for each programme is also available.

4.8.2 t) If you would like to discuss the outcomes of this trial issue, please contact UK NEQAS LI using the contact details provided.

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NACFC

NATIONAL ACADEMY
FOR CLINICAL FLOW CYTOMETRY

UK NEQAS for Leucocyte Immunophenotyping (UK NEQAS LI) based in the Royal Hallamshire Hospital, Sheffield Teaching Hospitals NHS Foundation Trust, the University of Sheffield and Alexion (UK) are proud to announce the formation of the National Academy of Clinical Flow Cytometry.

This facility has been founded on an unrestricted educational grant from Alexion (UK) and will be based at the University of Sheffield. The aims of the Academy are to provide tuition from international experts to Healthcare Scientists performing clinical flow cytometric analysis and to ensure that these tests are performed at the highest standard. The academy will therefore have a positive benefit for the treatment and management of thousands of patients across the UK and beyond.

Paroxysmal Nocturnal Haemoglobinuria (PNH) testing course

This specific course aims to train scientists in the flow cytometric detection and monitoring of Paroxysmal Nocturnal Haemoglobinuria (PNH). It is a rare disease, occurring at any age, with a prevalence of 2 cases per million and can be fatal if not detected and treated correctly.

This initiative will aim to improve detection and monitoring of PNH in clinical flow cytometry laboratories within the National Health Service.

It is envisaged that by improving both knowledge and skills of healthcare scientists there will be a dramatic improvement in the diagnosis and monitoring of PNH. This will lead to improved standardization of testing procedures at a National and ultimately International level.

Faculty

The National Academy, led by Professor David Barnett and his UK NEQAS LI team, will be based within the University of Sheffield. It has a faculty of internationally respected scientists such as Professor Robert Sutherland (Toronto, Canada), Andrea Illingworth (Bangor, USA) and Dr Steven Richards (HMDS, Leeds) who will teach clinical healthcare scientists practicing in UK hospitals on how to implement best practices for PNH testing.

Course details

Each course will be specifically targeted at an instrument type currently in use for clinical flow cytometry and will be delivered over 3 days by international experts in the field. All courses will feature classroom and practical sessions that will ensure that students receive the best tuition in the flow cytometric detection of PNH.

Registration

Via on-line form on our website at **www.sheffield.ac.uk/nacfc**

Registration for the course costs £100 and includes 2 nights' accommodation, all meals and pre- and post-course practical assessments.

Course dates

Becton Dickinson
Flow Cytometer Course
13–15 July
(BD FACSCalibur and BD FACSCanto II Practical Sessions)
Tutors: Prof. Rob Sutherland, Dr Steven Richards, Daniel Payne

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Beckman Coulter
Flow Cytometer Course
21–23 September
(BC FC500 and BC Navios Practical Sessions)
Tutors: Andrea Illingworth

Becton Dickinson
Flow Cytometer Course
26–28 October
(BD FACSCalibur and BD FACSCanto II Practical Sessions)
Tutors: Prof. Rob Sutherland, Daniel Payne

Beckman Coulter
Flow Cytometer Course
23–25 November
(BC FC500 and BC Navios Practical Sessions)
Tutors: Daniel Payne, Andrea Illingworth

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Sponsor

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Jointly hosted by

UK NEQAS
Leucocyte Immunophenotyping

The University Of Sheffield.

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